

and the amplitude of the reaction was used to monitor the time course of RNA binding. The kinetics of enzyme and RNA binding followed a two-step mechanism: an initial binding was followed by a conformational change. The enzyme and RNA binding proceeded to equilibrium about six times faster at 37 °C than at 30 °C, suggesting the conformational change involved in RNA binding is temperature sensitive. Following incubation of RNA and enzyme to form an active complex, we used chemical quench-flow methods to measure the ground state binding K_d and the incorporation rate of a correct nucleotide. The fidelity of the polymerase was determined by measurement of the incorporation of incorrect nucleotides. Using these methods, we characterized the Dengue polymerase in its elongation mode by measuring the kinetics of RNA and polymerase binding and the kinetics for nucleotide binding and incorporation. Based on these data, we built a working model for studying the selectivity of Dengue polymerase.

2337-Pos

Structure-Guided Design of Novel Inhibitors of Human Uridine Phosphorylase 1

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Uridine phosphorylase (UPP) is a key enzyme of pyrimidine salvage pathways, catalyzing the reversible phosphorylase of ribosides of uracil to nucleobases and ribose 1-phosphate. It also plays a role in the activation of pyrimidine-based chemotherapeutic compounds such as 5-fluorouracil (5-FU) and its prodrug capecitabine. In some cases, an elevated level of this enzyme in solid tumours is thought to contribute to the selective action of these drugs. Nevertheless, the therapeutic value of these fluoropyrimidine antimetabolites is often limited by their toxicity to normal tissue. To address this shortcoming, specific inhibitors of UPP, such as 5-benzylacyclouridine (BAU), have been clinically studied for their ability to moderate the cytotoxic side effects of 5-FU and its derivatives, so as to improve the therapeutic index of these agents. We have determined the high resolution structures of human uridine phosphorylase 1 (hUPP1) in complex with natural ligands and known inhibitors. The structures reveal important details underlying the architecture of hUPP1's active site and the proximate surfaces that influence binding of BAU and analogous acyclouridine compounds. This data provides opportunities for designing more potent inhibitors of this enzyme. For instance, the back wall of the substrate binding pocket is conformationally unique relative to earlier elucidated structures of microbial homologues of UPP. These features can be exploited to develop novel inhibitory compounds with improved efficacy against the human enzyme as a step toward the development of better chemotherapeutic regimens that protect normal tissues with relatively lower UPP activity.

Protein Structure II

2338-Pos

Factor Xa Dimerization and Prothrombinase Complex Formation are Competitive Process on a Membrane Surface

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Table of Contents

Exposure of phosphatidylserine (PS) molecules on activated platelet membranes is a crucial event in blood coagulation. Binding of PS to a specific site on factor Xa (fXa) and factor Va (fVa) promotes their assembly into a complex that dramatically enhances proteolytic activity of fXa. Recent studies demonstrate that by both soluble PS and PS-containing membranes promote formation of inactive fXa dimer at 5mM Ca^{+2} . The fluorescence anisotropy of active site labeled fXa, FEGR (Fluorescein-GLU-GLY-ARG-chloromethylketone)-Xa, is decreased in the presence of PS membrane on which it forms dimer. We report now the addition of fVa to membrane-bound FEGR-Xa produced fVa- FEGR-Xa complex formation with a K_d , surface approximately 60-fold lower than that characterizing FEGR-Xa surface dimerization, clearly indicating fVa strongly competed with fXa dimer formation in order to form active Xa-Va complex on the membrane surface. Analysis of FEGR-Xa fluorescence anisotropy yielded roughly constant K_d 's for Xa-Va interaction with increasing Ca^{2+} concentration from 2 to 5 mM Ca^{2+} despite the fact that fXa dimer formation varied dramatically over this Ca^{2+} range. Experiments performed at varying membrane and fVa concentrations for both 23 nm and 120 nm confirmed that protein distribution between vesicles was sufficiently rapid as to overcome any possible effects of membrane discreteness. We conclude that PS-induced fXa dimerization on membrane strongly competes with fXa-fVa complex formation at high Ca^{2+} concentrations. Supported by USPHS grant HL072827 to BRL.

2339-Pos

Sans and Osmotic Stress Approach to Study Protein Preferential Hydration and Association

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The function of biological macromolecules necessarily depends on their hydration and interactions. However, it can be challenging to appropriately and directly measure these forces. We are using a combined small-angle neutron scattering (SANS) and osmotic stress approach to directly correlate protein structure and structural transitions with the associated hydration and energetics. We performed SANS experiments on hexokinase (HK) to investigate protein preferential hydration by solute molecules, called osmolytes, and the interactions responsible for HK dimer formation. The HK monomer-dimer equilibrium plays a regulatory role but its importance to function is not entirely clear. With SANS, three regions of scattering contrast are created upon osmolyte addition: protein, protein-associated water, and bulk water/osmolyte solution. Changes in the zero-angle scattering intensity, $I(0)$, and the apparent radius of gyration, R_g , with increasing osmolyte concentration are used to quantify the number of osmolyte-excluding water molecules associated with protein. We observe the preferential hydration of HK monomer and dimer states to depend on the osmolyte chemistry and size but find the calculated hydration change accompanying the monomer-dimer transition to be independent of the osmolyte used. By experimentally exploring the forces that are important for guiding protein association, we hope to address critical questions concerning protein structure, hydration, and interactions.

2340-Pos

Understanding the Effects of Molecular Crowding on the Structure and Stability of Proteins Using NMR Spectroscopy

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Much of the research on biological proteins is performed *in vitro* (under artificial conditions) involving the isolation of the protein from the cell. The living cell, however, constitutes a very complex system, and a protein's structure and stability may be influenced by its native environment. An understanding of the effects of molecular crowding will provide important information regarding a protein's structure, dynamics, and stability *in vivo*.

This study involves the titration of ¹⁵N FGF-1 with various intracellular components (to simulate an in-cell environment) followed by NMR spectroscopy to determine any chemical shift perturbation corresponding to shifting amino acid residues. Fibroblast Growth Factor 1 (FGF-1) is a protein involved in cellular proliferation, wound healing, and cancer development and metastasis. Little information is known regarding FGF-1's interactions inside the cell, as it follows a non-classical secretion pathway. To better understand the role of intracellular proteins on the structure of FGF-1, several experiments were carried out using multi-dimensional NMR spectroscopy; to the FGF-1 sample were added (1) intracellular proteins (from the purification of unlabeled FGF), (2) intracellular proteins and lysozyme, and (3) lysozyme alone (added as a control). HSQC data was obtained at regular intervals and processed using XWIN-NMR and Sparky software. A chemical shift perturbation plot was constructed from the data to show the (individual and combined) effects of the addition of the intracellular proteins and/or lysozyme on FGF-1. The preliminary results of this study indicate that a moderate number of amino acid residues were perturbed with the addition of intracellular proteins. This implies that molecular crowding plays a role in the structural conformation of FGF-1 and possibly other proteins *in vivo*.

2341-Pos

Anthrax Protective Antigen Oligomerization Regulates Toxin Activity

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Anthrax toxin (Atx) is a key virulence factor secreted by *Bacillus anthracis*. This three-protein toxin includes protective antigen (PA) and two enzymatic components, lethal factor (LF) and edema factor (EF), which must assemble into oligomeric complexes to disrupt cell physiology. Atx complexes are endocytosed, where they convert to a transmembrane channel that transports LF and EF into the cytosol. Assembly from monomeric components may occur in two physiological contexts: 1) in the bloodstream and 2) on cell surfaces. We have previously shown that the assembly of toxin complexes on cell surfaces produces a mixture of ring-shaped homooctameric or homoheptameric PA oligomers in a 1:2 ratio, which assemble via dimeric PA intermediates. Here we investigate how Atx complexes assemble in bovine blood. We find that, under